UNIVERSITY OF Cincinnati

Biosafety Levels 1 and 2

Biological Laboratory Safety Manual

Principal Investigator:	
Building:	
Room Number(s):	

References:

1. Laboratory Biosafety Manual, 3rd edition, The World Health Organization, 2004
2. Biohazards in Microbiological and Biomedical Laboratories, 4th edition, Centers for Disease Control and Prevention, 1999
3. The NIH Guidelines for Research Utilizing Recombinant DNA Molecules.

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Emergency Contact Information

Biosafety Office	558-5210	Risk Assessments Incident Reporting
Medical Treatment During Business Hours	University Health Services 584-4457	Medical Evaluation Incident Reporting Post-incident follow-up and coordination of care
After Business Hours	University Hospital Emergency Department 584-5700	Emergency Medical Treatment
Environmental Health & Safety	556-4968	Report: Accidents, spills OSHA related issues, questions Chemical safety

For all other emergencies dial 911 for emergency response.

Foreward

The University of Cincinnati is committed to providing a safe work and academic environment. To ensure the safety of personnel, students, research participants, visitors, the public and the environment, the University has established departments with the specific tasks of identifying hazards and reducing or eliminating associated risks. These departments include Environmental Health and Safety, Institutional Biosafety, Radiation Safety, the Institutional Review Board and the Institutional Animal Care and Use Committee.

The University of Cincinnati Institutional Biosafety Committee (IBC) and Biological Safety Officer (BSO) are specifically charged with the review, approval and monitoring of all research activities in which recombinant DNA and biohazardous agents are utilized. The IBC is a volunteer committee that comprises a panel of scientific, medical and occupational health and safety experts to ensure that the risks of the proposed research are thoroughly evaluated and plans to minimize and manage those risks can be implemented.

The BSO reports to the Compliance Officer and the Vice President for Research. The BSO and staff are responsible for monitoring all research activities and implementing safe work practices to ensure employee and public health using the current best practices in biosafety as described (in some cases, mandated) by the National Institutes for Health, the World Health Organization and other United States departments and agencies. In addition, the BSO is responsible for ensuring compliance with regulations that apply to the use, possession or transfer of biohazardous agents.
The process of review, approval and monitoring is necessary but is not intended and should not be perceived to be a means to inhibit research. The priority of the IBC and BSO is to facilitate research activities while ensuring the safety of personnel and the public.

Sandra J. Degen, PhD
Vice President for Research

Introduction

Laboratory Biosafety

"Laboratory Biosafety" is the term used to describe the containment principles, technologies and practices that are implemented to prevent unintentional exposure to pathogens and toxins, or their accidental release.

All research or work at the University of Cincinnati involving the use of biohazardous agents and recombinant DNA must be reviewed and approved by the Institutional Biosafety Committee. This is mandated by the National Institutes of Heath ***Guidelines for Research Involving the Use of Recombinant DNA Molecules*** (NIH Guidelines) for any entity utilizing NIH funding. The Institutional Biosafety Committee is a volunteer committee consisting of a Chairman, the Biological Safety Officer, scientists and medical professional representing different relevant areas of expertise, animal care personnel and community representatives. The IBC's role is to review and approve research and develop institutional policies governing research.

Approved research and work is monitored by the University of Cincinnati Biosafety Department. In addition, the Biosafety Department serves as a resource to PIs, laboratory personnel and animal care personnel on safe work practices and procedures and ensures institutional and individual compliance with federal, state and local regulations governing the use of biohazardous agents. The Biological Safety Officer has responsibility for the overall implementation and day to day management of the University's biosafety program.

This laboratory biosafety manual contains the minimum precautions and procedures which are required in Biosafety Level 1 and Biosafety Level 2 laboratories. There are reserved sections which will contain additional practices and procedures for a particular laboratory or protocol. These additional practices will be determined by the Biological Safety Officer and/or Institutional Biosafety Committee upon review of a research proposal or during a post-approval risk assessment.
This manual does not address risks, procedures or practices related to research or work with Biosafety Level 3 agents or which require Biosafety Level 3 containment. Research or work which requires Biosafety Level 4 practices or containment is prohibited at the University of Cincinnati.

General Principles of Biological Safety

Risk Assessment

A risk assessment is the rational application of safety principles to available options for handling hazardous materials. The following characteristics are to be considered when evaluating use of a potential pathogen:

- Nature of agent (risk group)
- Source of agent
- Route of infection
- Dissemination of agent

Agent Risk Group

The biological and physical nature of human pathogens can be categorized into risk groups (RG) based on the transmissibility, invasiveness, virulence (i.e., ability to cause disease), and the lethality of the specific pathogen. Risk groupings of infectious agents (RG1 through RG4) generally correspond to biosafety levels (BL1 through BL4), which describe containment practices, safety equipment, and facility design features recommended for safe handling of these microorganisms. A parallel series of animal biosafety levels (ABSL1 through ABSL4) applies to handling of infected or potentially infected animals.

- **Risk Group 1** agents are not associated with disease in healthy human adults.
    - Corresponding Biosafety Level 1 which requires standard laboratory facilities and standard microbiological practices,
- **Risk Group 2** agents are associated with human disease which is rarely serious; treatment is usually available.
    - Corresponding Biosafety Level 2 which requires more sophisticated personal protection and engineering controls (e.g., facilities and equipment) than are available in standard laboratories, as well as special handling and decontamination procedures.
- **Risk Group 3** agents are associated with serious or lethal human disease; treatment may be available; low community risk.
    - Corresponding Biosafety Level 3 which requires very sophisticated personal protection, specialized facilities and engineering controls; as well as special handling and decontamination procedures.
- **Risk group 4** agents are associated with serious or lethal human disease; treatment is not usually available, high community risk.
    - Not currently permitted for use at the University of Cincinnati.

- Consideration must also be extended to **microorganisms that cause diseases in animals and plants**, which are not categorized like human pathogens into risk groups.

- **Attenuated strains** should be handled with the same precautions as the virulent strain unless the reduced pathogenicity is well documented and is irreversible.

- **Viral vectors**, even if rendered replication defective, still may pose a threat of recombination with wild-type strains and/or unintentional delivery of their foreign genes.

Consideration of the risk group, however, is merely a starting point for the comprehensive risk assessment. Other factors to be considered are:

Source of Agent

The source or host of the agent must also be considered when conducting a risk assessment for potential use of a pathogen. Determining the source of the agent will assist in determining the proper equipment and procedures required for safe research.

Clinical and Diagnostic Specimens

Any specimen from people or animals has the potential to contain infectious agents. Personnel in laboratories and clinical areas handling human blood or body fluids must practice universal precautions, an approach to infection control wherein all human blood and certain human body fluids are treated as if known to be infectious for human immunodeficiency virus (HIV), hepatitis B virus (HBV), and other blood-borne pathogens. Clinical and diagnostic specimens include, but are not limited to, the following:

- Blood
- Tissues
- Organs
- Semen
- Vaginal secretions
- CSF
- Synovial fluid
- Pleural fluid
- Saliva from dental procedures
- Amniotic fluid
- Pericardial fluid

Cell Cultures

Routine manipulations of cell cultures have the ability to generate aerosols. These activities include, but are not limited to, the following:

- Centrifugation
- Sonication
- Pipetting
- Shaking
- Spills
- Cell sorting
- Inoculation
- Re-suspension of lyophilized samples

Equipment used for manipulations of infectious materials must be evaluated to determine the need for secondary containment. A certified biological safety cabinet should be used for all activities that have the potential to generate aerosols. If a particular procedure makes the use of a cabinet impractical, an alternate method of secondary containment must be considered.

Animals

Numerous risks may be present when animals are used in studies of microorganisms, as well as for studies of hazardous chemicals. These risks include, but are not limited to, the following:

- Inoculation from animal bites and scratches
- Exposure to animal excreta in cage bedding
- Exposure to animal allergens
- Self-inoculation from instruments and sharps
- Generation of aerosols during procedures
- Preparation and use of hazardous chemicals

The use of personal protection and secondary containment must be considered when using animals for research.

Plants

Plants, as do animals, pose a potential threat to human safety. These risks include, but are not limited to, the following:

- Inoculation from scratches
- Exposure to allergens and plant toxins
- Self-inoculation
- Generation of aerosols
- Preparation and use of hazardous chemicals

The use of personal protection and secondary containment must be considered when using animals for research.

Select Biological Agents and Toxins

The US Department of Health and Human Services (HHS) and the US Department of Agriculture (USDA) have developed a list of select biohazardous agents and toxins that have the potential to pose a severe biosecurity threat to public health, animals, and agricultural crops. As directed by the US Patriot Act, HHS and USDA have adopted strict regulations for the obtaining, possession, use, or transfer of any of these selected agents. Failure to comply with the established regulations can result in significant civil and criminal penalties.

Any investigator considering the use of select agents or toxins must contact the University Biosafety Officer (Gary E. Dean, PhD, 513-558-0065) to discuss the specifics of the requirements. HHS regulations in 42 CFR Part 73 Possession, Use, and Transfer of Select Agents and Toxins and the companion USDA regulations in 9 CFR Part 121 require federal registration and inspection; restricted lab access; development of written and strictly followed safety and security plans; personnel background checks by the FBI (including fingerprinting); specialized training; strict recordkeeping and reporting of agent use, transfer, loss, or destruction.

In determining whether to use select agents, researchers are encouraged to give careful consideration to the personal responsibilities, financial costs, and lengthy application and permit process involved with compliance. Any plans for use of select agents could easily take several months to get the appropriate permits and approvals and establish the security and protocols

necessary to comply with the regulations. Sources of research funds to cover the cost of facility security improvements will need to be identified.

There is a small quantity exemption available for some of the select toxins. If the aggregate amount of toxin in the possession of a researcher can be kept below the specified exempt quantity, most of the rules do not apply. It should be noted that even when taking advantage of the small quantity exemption, the investigator is required to establish an inventory system to ensure the limit is not exceeded. Regardless of the amount, the University Biosafety Officer must be contacted prior to beginning work

The US Government website for Select Biological Agents and Toxins: www.selectagents.gov, including the list of agents, exclusions and exemptions:
http://www.selectagents.gov/agentToxinList.htm

Routes of Infection

Risk assessments must also consider the potential route of infection for the agent in question. The assessment must be based on potential ways a laboratory worker can be infected with a given agent. Not only must the natural state of the micro-organism be considered, but the future state caused by manipulation or physical activities (i.e., aerosols, splashes, etc.)

Oral/Gastrointestinal

- Eating, drinking and smoking in the laboratory
- Mouth pipetting
- Transfer of microorganisms to mouth by contaminated fingers or articles

Cutaneous

- Inoculation with a hypodermic needle, other sharp instrument or glass
- Cuts or scratches

Eyes/Mucosal Membranes

- Splashes of infectious material into eyes or nose
- Transfer of microorganisms to eyes or nose by contaminated fingers

Respiratory

- Inhalation of aerosolized microorganisms

Dissemination of Agent

Many tasks performed inside research laboratories may disseminate biohazardous agents and put people at risk of exposure. The risk assessment must consider the tasks to be performed and what measures must be taken to mitigate or eliminate potential risks. These tasks include, but are not limited to, the following potential hazards:

- Opening/handling of any packages or containers (including flasks and plates) that contain live biohazardous agents including visual checks for growth and contamination in sealed containers

- Transfer of cultures to agar slants, plates, or liquid media

- The transfer or transportation of biohazardous materials into and out of equipment, rooms, buildings, etc.
- Biochemical, biological, genetic, or physical testing of isolates to determine and/or confirm speciation and/or drug sensitivity
- Preparing, freezing, and thawing stock cultures
- Harvesting cells and culture filtrate
- Sterilization of contaminated glassware and supplies used in lab procedures
- Routine cleaning and maintenance of biological safety cabinets, equipment and laboratory space
- Purification of proteins, DNA, sub-cellular fractions, and DNA electroporation
- In-vitro immunological assays, such as T-cell proliferation, macrophage activation, etc.
- Examination or colony counting of solid agar plates
- Preparing and staining fixed slides
- Labeling with radioactive compounds
- Cleaning animal cages/changing animal bedding
- Isolation/extraction of animal tissues
- Homogenization of animal tissues
- Infecting/injecting animals

Safety Considerations

Teaching Laboratories

Whenever possible, the use of avirulent strains of infectious microorganisms in teaching laboratories is strongly recommended. However, even attenuated microbes must be handled with care. Students must be cautioned against and trained to prevent unnecessary exposure, as exposure to “avirulent” strains may be problematic in the immuno-compromised individual. Establishment of safety consciousness is essential to the conduct of good science.

Research Laboratories

Experiments in research laboratories using high concentrations or large quantities of pathogens increase the risk of exposure and the possibility of overcoming natural barriers to infection. The use of animals in research on infectious diseases also presents greater opportunities for exposure.

Clinical Laboratories

Personnel in laboratories performing diagnostic tests of clinical specimens from human or animal patients are often at risk of exposure to infectious agents. The absence of an infectious disease diagnosis does not preclude the presence of pathogens. This is especially true of materials from patients who receive immunosuppressive therapy, since such treatment may activate latent infectious agents.

Health Status

Circumstances will sometime warrant special considerations or measures to prevent infection of laboratory personnel by microorganisms. Certain medical conditions increase your risk of potential health problems when working with pathogenic microorganisms and/or animals. These include:

- Pregnancy
- Immuno-suppression (HIV+, medications, illness, age, etc.)
- Animal-related allergies

By law, no one who is qualified and authorized can be excluded from working in a laboratory. Additionally, the law mandates that workers do not have to disclose their medical conditions to their supervisors/employers. To avoid possible risks, however, an open and honest line of communication should be opened between the worker and their supervisor. It is recommended that if the worker suffers from a particular medical condition that they discuss it with their family physician for guidance and monitoring.

Biohazard Containment

Although the most important aspect of biohazard control is the awareness and care used by personnel in handling infectious materials, certain features of laboratory design, ventilation, and safety equipment can mitigate dissemination of pathogens and exposure of personnel should an accidental release occur.

Practices and Procedures

The following practices are important not only for preventing laboratory infection and disease, but also for reducing contamination of experimental material. The basic practices and procedures provide a progressive foundation for work with agents that pose an inherently higher risk.

Biosafety Level 1 (BSL1)

BSL1 is suitable for work involving well-characterized agents not known to consistently cause disease in healthy adult humans, and of minimal potential hazard to laboratory personnel and the environment. The following standard and special practices, safety equipment and facilities apply to agents assigned to Biosafety Level 1:

Standard Microbiological Practices

- Access to the laboratory is limited or restricted at the discretion of the laboratory director when experiments or work are in progress.
- Persons must wash their hands after handling viable materials, after removing gloves, and before leaving the laboratory.
- Eating, drinking, smoking, handling contact lenses, applying cosmetics, and storing food for human use are not permitted in the work areas. Persons who wear contact lenses in laboratories should wear goggles or a face shield.
- Food is stored outside the work area in cabinets or refrigerators designated and used for this purpose only.
- Mouth pipetting is prohibited
- Policies for the safe handling of sharps are instituted.
- All procedures are performed carefully to minimize the creation of splashes or aerosols.
- Work surfaces are decontaminated at least once a day and after any spill of viable material.
- All cultures, stocks, and other regulated wastes are decontaminated before disposal by an approved decontamination method. Materials to be decontaminated outside of the immediate laboratory are to be placed in a durable, leak-proof container and packaged in accordance with applicable local, state, and federal regulations before removal from the laboratory.
- The laboratory must participate in the University insect and rodent control program.

Special Practices

None

Safety Equipment (Primary Barriers)

- Special containment devices or equipment, such as a biological safety cabinet, are generally not required for manipulations of agents assigned to BSL1.
- It is recommended that laboratory coats, gowns, or uniforms be worn to prevent contamination or soiling of street clothes.
- Gloves should be worn if the skin on the hands is broken or if a rash is present. Alternatives to powdered latex gloves should be available.
- Protective eyewear should be worn for conduct of procedures in which splashes of microorganisms or other hazardous materials is anticipated.

Laboratory Facilities (Secondary Barriers)

- Each laboratory contains a sink for handwashing.
- The laboratory is organized so that it can be easily cleaned.
- Carpets and rugs in laboratories are prohibited.
- Bench tops are impervious to water and are resistant to moderate heat and the organic solvents, acids, alkalis, and chemicals used to decontaminate the work surface and equipment.
- Laboratory furniture is capable of supporting anticipated loading and uses. Spaces between benches, cabinets, and equipment are accessible for cleaning.

Biosafety Level 2 (BSL2)

BSL2 adopts the same basic safety principles as outlined in BSL1, but provides for additional worker and environmental protection. BSL2 involves work with agents of moderate potential hazard to personnel and the environment. It differs from BSL1 in that:

- Laboratory personnel have specific training in handling pathogenic agents and are directed by competent scientists
- Additional precautions are taken with contaminated sharp items
- Procedures in which infectious aerosols or splashes may be created are conducted in biological safety cabinets or other physical containment equipment.

The following practices, safety equipment, and facilities apply to biohazardous agents assigned to BSL2:

Standard Microbiological Practices

See BSL1 Standard Practices

Special Practices

- The laboratory director establishes policies and procedures whereby only persons who have been advised of the potential hazards and meet specific entry requirements may enter the laboratory.
- The laboratory director must advise all who enter of the potential risks to pregnant and immuno-compromised personnel. Personnel who may be at risk should consult with their family physician before entering.
- A biohazard sign, along with contact information, must be posted on the entrance to the laboratory when BSL2 agents are in use. Information must include the agent(s) in use, the biosafety level, the required immunizations, the investigator's name and telephone number, any personal protective equipment that must be worn in the laboratory, and any procedures required for exiting the laboratory.
- Biosafety procedures are incorporated into standard operating procedures or in a biosafety manual adopted or prepared specifically for the laboratory by the laboratory director. Personnel are advised of all hazards and are required to read and follow instructions on practices and procedures.
- The laboratory director ensures that laboratory and support personnel receive appropriate training on the potential hazards associated with the agent(s) involved, the necessary precautions to prevent exposures, and the exposure evaluation procedures. Personnel receive annual updates or additional training as necessary for procedural or policy changes.
- A high degree of precaution must always be taken with any contaminated sharp items, including needles and syringes, slides, pipettes, capillary tubes, and scalpels.
- Needles and syringes or other sharp instruments should be restricted in the laboratory for use only when there is no alternative, such as injection, phlebotomy, or aspiration of fluids.
- Plasticware should be substituted for glassware whenever possible.
- Only needle-locking syringes or disposable syringe-needle units (i.e., needle is integral to the syringe) are used for injection or aspiration of infectious materials.

Used needles must not be manipulated by hand before disposal; rather, they must be carefully placed in approved sharps containers.

- Non-disposable sharps must be placed in a hard-walled container for transport to a processing area for decontamination.
- Syringes which re-sheathe the needle or needle-less systems should be used when possible.
- Broken glassware must be removed only by mechanical means such as a brush and dustpan, tongs, or forceps.
- Containers of contaminated needles, sharp equipment, and broken glass are decontaminated before disposal in accordance with local, state, and federal regulations.
- All potentially infectious materials are placed in a container with a cover that prevents leakage during collection, handling, processing, storage, transport, or shipping.
- Laboratory equipment and work surfaces should be decontaminated on a routine basis, after work with infectious materials, and especially after spills, splashes, or other contamination by infectious materials.
- Contaminated equipment must be decontaminated in accordance with local, state, or federal regulations before it is sent for repair, maintenance, or disposal.
- Spills and accidents that result in exposures to infectious materials are immediately reported to the laboratory director. Medical evaluation, surveillance, and treatment are provided as appropriate and written records are maintained.
- Animals not involved in the work being performed are not permitted in the laboratory.

Safety Equipment (Primary Barriers)

Primary barriers for work at BSL2 include all the requirements outlined for BSL1, but with additional measures focused on micro-organism containment and worker protection. These additional measures include the following:

Properly maintained biological safety cabinets, preferably Class II, and/or other appropriate personal protective equipment or physical containment devices must be used whenever:

- Procedures with a potential for creating infectious aerosols or splashes are conducted (See Dissemination of Agents under Risk Assessment).

- High concentrations or large volumes of infectious agents are used. Such materials may be centrifuged in the open laboratory only if sealed rotors or centrifuge safety cups are used and if these rotors or safety cups are opened only in a biological safety cabinet.

Additional information regarding biosafety cabinets can be found below.

Laboratory workers must also wear personal protective equipment (PPE) while working in a BSL2 laboratory. The level of protection may vary slightly betweens agents, but must include the following:

- Protective laboratory coats, gowns, smocks, or uniforms designated for lab use are worn while in the laboratory. Protective clothing is removed and left in the laboratory before leaving for non-laboratory areas.
- All protective clothing is either disposed of in the laboratory or laundered. It should never be taken home by personnel.
- Gloves are worn to prevent contact with potentially infectious materials, contaminated surfaces or equipment. Gloves are disposed of when contaminated and removed when work with infectious materials is completed or when the integrity of the glove is compromised. Gloves are not to be reused.
- Alternatives to powdered latex gloves should be available.
- Hands are washed following removal of gloves.
- Face protection (goggles, mask, or face shield) is used when the potential for splashes or sprays of infectious materials exist.

Additional information regarding PPE is discussed below.

Laboratory Facilities (Secondary Barriers)

Secondary barriers for work at BSL2 include all the requirements outlined for BSL1, but with additional measures focused on security and workplace environmental protection. These additional measures include the following:

- Provide lockable doors for facilities that house restricted agents.
- Consider locating new laboratories away from public areas.
- Chairs and other furniture used in laboratory work should be covered with a non-fabric material that can be easily decontaminated.
- Install biological safety cabinets in such a manner that fluctuations of the room supply and exhaust air do not cause the biological safety cabinets to operate outside their parameters for containment.
- Locate biological safety cabinets away from doors, from windows that can be opened, from heavily traveled laboratory areas, and from other potentially disruptive equipment so as to maintain the biological safety cabinets' air flow parameters for containment.
- An eyewash station is readily available.
- Illumination is adequate for all activities, avoiding reflections and glare that could impede vision.
- There are no specific ventilation requirements. However, planning of new facilities should consider mechanical ventilation systems that provide an inward flow of air without recirculation to spaces outside of the laboratory.

Personal Protective Equipment

Any personal protective equipment (PPE) that has been used may NOT be worn outside the laboratory. Bringing used PPE into non-designated work areas may increase the risk of potentially spreading contamination. All used PPE must be kept in the laboratory or in a designated storage area immediately adjacent to the work area.

Protective Clothing

Protective clothing provides a physical barrier between the agent and the laboratory worker and can either be reusable or disposable. The type of protective clothing must be based upon the level of risk involved with the laboratory work.

Reusable clothing typically consists of:

- Laboratory coats
- Surgical scrubs/gowns
- Synthetic over-garments

Laboratory coats and scrubs offer convenient and low-cost laboratory protection. They can be easily stored when not in use and can be laundered frequently. Reusable clothing, however, is typically not impermeable to liquids (unless synthetic garments such as Tyvek are used) and can be difficult to decontaminate. Additionally, laboratory coats and scrubs are easily forgotten and worn outside of the work area.

Disposable clothing typically consists of:

- Hair and shoe covers
- Synthetic over-garments
- Protective sleeves

Disposable clothing eliminates the need for laundering and post-use storage. Additionally, it significantly reduces the possibility of secondary contamination, provides impermeability to liquids, and typically offers better protection from biohazardous agents. Disposable clothing, however, can cost more and may be time-consuming to don.

Gloves

Gloves provide primary protection for the most commonly exposed part of the laboratory worker—the hands. Gloves must be worn whenever there is the potential for contact with biohazardous materials and should never be reused. Latex and nitrile are the most common types of gloves used for working with biohazardous agents. A large variety of types of gloves exist for a variety of different applications, however, and when selecting a particular type of glove, the following factors must be considered:

- Allergies of laboratory personnel
- Length of cuff
- Thickness of material
- Use of chemicals

Gloves must be changed whenever a spill occurs or when a breach in the glove material is discovered.

The use of gloves can be eliminated when transporting materials outside of the laboratory if the material is placed in a secondary container and the outer surface is decontaminated.

Eye Protection

As the surface membrane of the eye is extremely vulnerable to biological infection, eye protection must always be worn in the laboratory. Eye protection includes:

- Goggles
- Safety glasses
- Face/splash shield

For non-aerosol generating activities, safety glasses and face shields provide adequate protection against splashes and spills inside the work area. They, however, will not provide substantial protection against airborne particles and aerosols.

Goggles offer increased protection (by protecting the sides and being somewhat air-tight) against spills and aerosol generating activities.

Contact lenses may be worn with discretion and in combination with eye protection.

Respiratory Protection

Before using any type of respiratory protection, consult EH&S for guidance (513-556-4968).

Respiratory protection is not required when working with most BSL1 and BSL2 agents. Respiratory protection is required, however, for some BSL2 agents when there is risk of aerosol generation that cannot be mitigated through the use of alternative procedures or containment equipment.

Respiratory protection can be provided by either a mask worn over the nose and mouth (N95) or a hood worn over the entire head (PAPR).

Mask

The most common type of mask is the N95 respirator, which has the following characteristics:

- Fits over nose and mouth
- Secured to head via elastic bands
- Fitted with HEPA filter
- Must be worn with safety glasses/goggles
- Disposable
- Low cost

N95 respirators must not be reused and are only as effective as the degree of "fit" to the worker's face. Head size, face size, and facial hair can all affect the fit of the mask and reduce the efficacy of protection.

Hood

The most common hood protection is the PAPR (powered air purifying respirator) which has the following characteristics:

- Hood covers entire head
- Blower unit worn on belt (connected to hood via a breathing tube)
- HEPA filtered air supply
- Positive air pressure
- Offers more protection than N95
- Can be re-used

PAPRs are more expensive than the N95 but offer greater respiratory protection and do not require the user to wear eye protection. The units tend to be bulky, but offer an effective alternative to those who can not achieve a proper N95 fit.

Surgical masks (unless fitted with a HEPA filter) do NOT provide respiratory protection against biohazardous agents.

N95s and PAPRs will NOT provide protection against volatile or dangerous chemicals and fumes.

Engineering Controls

Laboratory Ventilation

For containment in a laboratory to be effective, it is important that laboratory air pressure be lower (more negative) than that in the adjacent spaces. The negative air pressure differential ensures that air will enter the laboratory and not egress outward. To maintain proper negative room pressure the following points must be considered:

- Laboratory doors must be kept closed in BSL2 laboratories.
- The room should be located away from high-traffic areas
- Exhaust air from laboratories must not re-circulate into adjacent areas (unless HEPA filtered first)
- Exhaust systems should be hard-ducted to the outside

Ventilation Equipment

The biosafety cabinet is the most common type of equipment used for work with biohazardous agents. Not all equipment, however, is suitable for this line of work. Fume hoods and clean benches, which can closely resemble biosafety cabinets in appearance, are NOT suitable ventilation alternatives for work with biohazardous agents.

Chemical Fume Hood

Fume hoods are required when volatile or dangerous chemicals are used in the laboratory. They are NOT effective for use with biohazardous agents. Fume hoods offer the following characteristics:

- Only protect personnel (not product or outside environment)
- Exhaust unfiltered air to the outside
- Unfiltered air from the laboratory is drawn into the hood.

Clean Bench

Clean benches provide ONLY product protection by creating a unidirectional airflow generated through a HEPA filter. The discharged air flows across the work surface and directly into work area. Clean benches are NOT suitable for work with biohazardous agents.

Biological Safety Cabinets

Biological safety cabinets (BSC) are designed to contain biological hazards and to allow products to be handled in a clean environment. They ARE suitable for work with biohazardous agents. BSCs have the following characteristics:

- Inward airflow for personal protection
- HEPA-filtered exhaust air for environmental and personal protection
- HEPA-filtered supply air for product protection (except Class

Use of Biological Safety Cabinets

Location

The integrity of the airflow into the BSC is fragile and can be easily disrupted by air currents generated by nearby doors and high traffic areas, which can compromise protection to personnel and the environment. As such, BSCs must be located several feet away from doorways and high traffic areas. Additionally, 12 inches of clearance on the sides and 18 inches on the top must be maintained to provide for accurate measurements and filter changes.

Operation and Maintenance

BSC are designed to operate 24 hours per day. By keeping the exhaust fan operating at all times, the potential for contamination inside the cabinet is reduced. Additionally, continuously turning the fan on and off will decrease the life of the motor. Turning off the fan, however, will NOT release infectious particles from the filters.

The front sash can be placed in the closed position when not in use. It will not affect the circulation of air nor will it place additional strain on the fan motor.

Certification and Maintenance

Any maintenance or certification of a BSC must be done by a nationally certified technician. The following must be considered when planning for certification or maintenance:

- Re-certification must be done on an annual basis
- The cabinet must be thoroughly decontaminated before any repairs or movement

Cleaning and Disinfection

The BSC must be wiped down with disinfectant prior to use. This will reduce the possibility of cross contamination from environmental sources or from other users. All surfaces, inside and out, must be wiped down, allowing for the minimum contact time for that particular disinfectant.

Make note of the properties of disinfectant as many will corrode the stainless steel surface of the cabinet. This can be reduced by a follow-up wipe down with water or ethanol.

After work is completed, the same cleaning procedure must be followed. Leave the work space clean for the next worker.

UV Lights

The efficacy of UV light in BSCs is debatable. Most studies indicate that the use of UV light in not an effective method of BSC decontamination. Therefore, it is not recommended for use.

Alarms

A BSC alarm indicates a possible malfunction of the cabinet and must be handles immediately to prevent worker contamination.

- The sash alarm indicates that the front sash is opened more than 6-8 inches in height. The alarm will stop once the sash is returned to the proper height.
- An airflow alarm indicates that proper flow of air inside the cabinet has been interrupted. The cabinet must NOT be used and a technician should be called immediately.

Use

The worker's movements are the primary cause of airflow disturbance in BSCs. The following must be considered when working in a BSC:

- The worker must be positioned directly facing the front of the cabinet in a comfortable, seated position.
- Arms must be moved slowly, straight in and out of the cabinet, avoiding any sideways sweeping motions.
- Hand and arm movements inside the cabinet must be deliberate and flow from one side to the other.
- The elbows must never rest on the front grill.
- Conducting work on an absorbent pad will reduce the potential for contamination should a spill occur.

PPE

The PPE required for work at the bench must be used for work in the cabinet. While extending arms inside the cabinet, the shortening of protective clothing must NOT expose the skin between the sleeve and the top of the glove. Masking tape can be used to tape the glove to the sleeve or extended cuff gloves can be used, placing the cuff over the sleeve.

Materials Placement

Materials placement and work must not begin for approximately 5 minutes after disinfection preparation. This will allow sufficient time for the inside air to be filtered and for air currents to stabilize.

The proper placement of work materials will significantly increase safe and effective use of the BSC.

- All materials needed for the experiment (as space permits) must be placed inside the cabinet before work is begun
- Materials must be superficially decontaminated prior to placement
- Materials must be placed according to their use ("clean" materials on one side, "dirty" materials on the other)
- Materials must never be placed on the front or rear intake grill

- Open flames must be minimized to reduce explosion hazards
- Place aerosol generating equipment near the rear of the cabinet

Spills

Refer to the EH&S website for the required components of a laboratory spill clean up kit: http://www.ehs.uc.edu/spill_kit_requirements.asp

Spills are considered the leading cause of potential worker contamination inside a BSC. A "spill" can be considered any unintended event where biohazardous material escapes its container. A spill can range from knocking over a beaker full of liquid to droplets leaking out of a pipet tip. Spills must be cleaned up immediately.

For purposes of this manual, spills can be classified as either small (<50mL) or large (>50mL), but the principles of response are the same.

Small Spills

For spills occurring in the BSC of less than approximately 50mL, the worker should do the following:

- Cease all work
- Place an absorbent/paper towel on the spill to contain it
- Gently pour or spray disinfectant on the towel (until saturation)
- Allow to sit for 1 minute (or the minimum contact time)
- Place towel in biohazard bag (inside BSC)
- Spray affected area with disinfectant
- Using a clean towel, wipe up spill area in a circular motion, starting from the perimeter, working inwards
- Place towel in biohazard bag
- Spray any affected supplies or equipment with disinfectant and wipe off
- Change gloves
- Spray any potentially affected areas of PPE with disinfectant
- Continue work

Large Spills

For spills occurring in the BSC of more than approximately 50mL, the worker should do the following:

- Cease all work
- Inform other lab workers that a spill has occurred
- Place absorbent towels over spill area
- Gently pour or spray disinfectant on the towel (until saturation)
- Spray any affected supplies or equipment with disinfectant
- Allow to sit for 15 minutes
- Change gloves
- Exposed PPE should be sprayed and replaced
- Place towels in biohazard bag (inside BSC)

- Thoroughly spray affected area with disinfectant
- Using a clean towel, wipe up spill area in a circular motion, starting from the perimeter, working inwards
- Place towel in biohazard bag
- Spray ALL supplies and inside of BSC with disinfectant and wipe off
- Change gloves
- Continue work
- Report spill to supervisor

Disposal of Biohazardous Waste

The Biological Safety Officer is responsible for ensuring the proper and safe disposal of biohazardous wastes (solid, liquid, infectious, non-infectious, sharps and recombinant DNA wastes).

Environmental Health and Safety is responsible for ensuring the proper final disposal of biohazardous wastes and the management of all chemical and biohazardous wastes at the University of Cincinnati. http://www.ehs.uc.edu/wastedisposal.asp

Methods of Decontamination

Biohazardous waste must be decontaminated before the end of each working day and before final treatment and disposal. Decontamination is the process of reducing the number of disease-producing microorganisms and rendering an object safe for handling.

The appropriate method to eliminate or inactivate a biohazard depends largely on the treatment equipment available, the target organism, and the presence of interfering substances (e.g., high organic content) that may protect the organism from decontamination. Other common factors that influence the efficacy of disinfection are contact time, temperature, water hardness, and relative humidity.

Various treatment techniques are available, but practicality and effectiveness govern which is most appropriate. The efficacy of the selected method against the particular biohazard must be documented by reference to accepted procedures or quantitative testing.

Special consideration must be given to treating waste that is co-contaminated with volatile, toxic, or carcinogenic chemicals, radioisotopes, or explosive substances. A particular treatment method may yield an additional hazard.

Steam Sterilization

Decontamination is best accomplished by steam sterilization in a properly functioning autoclave that is routinely monitored with a biological indicator. Indicator tape provides assurance only that a high temperature was reached; it does not indicate the proper pressure or time. Autoclave bags must be sealed (steam WILL penetrate the bag) to prevent spillage of materials. Non-steam autoclaves are not a reliable method of decontamination.

The University of Cincinnati is not licensed to utilize steam sterilization as a final treatment prior to disposal; therefore, all BSL2 biohazardous waste must be disposed of in red biohazard waste bags and barrels for removal and eventual incineration as the final treatment by a licensed transport and treatment facility. It is the recommendation of the Biosafety Office that all waste be autoclaved prior to disposal, in accordance with Ohio EPA guidelines, for hygiene purposes and to eliminate odors.

Chemical Surface Decontamination

Alcohols

Ethanol and isopropanol are effective against some vegetative forms of bacteria, fungi, and hydrophobic (enveloped) viruses, but will not destroy spores or hydrophilic viruses. The recommended strength is 70–90%. Alcohol can also be used for disinfection of instruments or surfaces that have low organic burden.

Alcohol-based hand-rubs are recommended for the decontamination of lightly soiled hands in situations where proper hand-washing is inconvenient or impossible.

Quaternary Ammonia

These disinfectants kill most fungi and vegetative gram positive bacteria but lack efficacy against mycobacteria, spores, and some viruses (including adenovirus). Quaternary ammonium compounds generally have low toxicity and irritancy and are relatively inexpensive. Commonly used disinfectants include HB Quat™, Roccal™, and Solucide™.

Aldehydes

Formaldehyde and glutaraldehyde have broad germicidal activity, but toxicity to humans limits their usefulness as laboratory disinfectants. Commonly used disinfectants include Cidex™ and Wavicide-01™.

Peroxides

Peroxygen compounds provide a wide range of bactericidal, viricidal, and fungicidal activity, although activity is variable against bacterial spores and mycobacteria. Corrosivity, cross reactivity, and explosive concentration levels must be considered. A commonly used disinfectant is Virkon™ .

Ethylene Oxide

Ethoxy compounds provides effective treatment of heat sensitive equipment. Ethylene oxide, however, is a known human carcinogen and release of ethylene oxide gas is restricted under federal and state regulations.

Halogens

Halogens are inexpensive and highly effective for decontamination of most biohazardous materials. Their drawbacks include short shelf life, easy binding to nontarget organic substances, corrosiveness, and skin reactivity. Solutions must be stored in an opaque bottle to reduce decay during storage and be made fresh on a weekly basis.

Household Bleach (sodium hypochlorite)

Fresh bleach (e.g. Clorox™) contains ~5.25% sodium hypochlorite. It is the least expensive, most convenient and most efficacious method to use for chemical decontamination. A solution of 20% household bleach in water is effective for surface decontamination; however, this should be followed by an ethanol or water rinse. Liquid biohazardous wastes should be diluted to a final concentration of 20% household bleach before final disposal.

Iodophors, complexes of iodine and carrier, have good germicidal properties with relatively low toxicity and irritancy. Efficacy has been demonstrated against most bacteria, viruses, and fungi (although contact time may vary). Commonly used disinfectants include Povidine™ and Betadine™.

Solutions containing halogens must not be autoclaved in a sterilizer that does not utilize a vacuum system. Additionally, halogen solutions containing radioactive iodine must not be autoclaved.

Phenols

Phenolic compounds are effective against vegetative bacteria, particularly gram positive species, and enveloped viruses, but not against spores. Phenols may also be used in combination with detergents for one-step cleaning and disinfection of surfaces. Phenol based disinfectants maintain their activity in the presence of organic material and are generally considered safe. Commonly used disinfectants include Vesphene™ and LpH™.

It is important to be aware that common laboratory disinfectants can pose specific hazards to the users. Ethanol and quaternary ammonium compounds may cause contact dermatitis. Chlorine in high concentrations can irritate the mucous membranes, eyes, and skin. The toxicity of aldehydes can limit their usefulness.

Large-volume areas such as fume hoods, biological safety cabinets, or rooms may be decontaminated using gases such as formaldehyde and ethylene oxide. These gases, however, must be applied with extreme care and only licensed personnel may perform such a decontamination method.

Exposure Response

For any possible or identifiable exposure to a hazardous substance, seek immediate medical assessment from either University Health Services (Monday through Friday, 7:30 a.m. to 4:30 p.m) or the University Hospital Emergency Department.

The best approach is to implement a well-prepared exposure response plan, regardless of immunization status, and to provide training to personnel according to this plan. The basic elements of a plan should include:

- A description of the agent
- Signs and symptoms of infection
- Characteristics of the agent, including:
    - Antibiotic resistance
    - Transmissibility
    - Atypical tissue tropism

    - Altered pathogenicity
- Emergency notification procedures

Tests and serum baselines to establish a history of exposure prior to work with the agent and periodically thereafter may be appropriate for a few pathogens such as Mycobacterium tuberculosis.

Transport of Dangerous Goods

The transportation of dangerous goods is regulated by U.S. Department of Transportation and the International Air Transport Association. The employer must certify training and the training must cover function specific aspects, safety, security, and general awareness. The requirements for proper packaging, labeling, marking and declaration apply when a commercial carrier provides service to transport the hazardous materials.

There are times when University employees transport biological materials, some of which meet the regulatory definition of a dangerous good, between buildings on the main campus or to outlying areas in an institutional vehicle. It may be impractical to hire a commercial carrier and it can be done safely with advance planning. This activity does not meet the regulatory definition of transportation in commerce and is allowed under the regulations. The regulatory standard of care still applies and you should follow virtually all of the procedures that you would use when employing a commercial carrier (you would not need a dangerous goods declaration, nor would you use the institution's contract for emergency response information).

The following steps should be used when transporting infectious substances without involving a commercial carrier:

- Use UN certified packaging that is appropriately marked and labeled.
- Prepare an MSDS-equivalent for the pathogen that will accompany the shipment.
- Train the worker about nature of the pathogen and emergency procedures.
- Have an emergency response kit (e.g., absorbent, disinfectant, biohazard bags) available.

Laboratory Bio-security

Traditional laboratory biosafety guidelines have emphasized use of optimal work practices, appropriate containment equipment, well-designed facilities, and administrative controls to minimize risks of infection or injury and to prevent contamination of the outside environment. Although many laboratories contain dangerous biologic, chemical, and radioactive materials, to date, only a limited number of reports have been published of materials being used intentionally to injure laboratory workers or others. Concern, however, has increased regarding possible use of biologic, chemical, and radioactive materials as terrorist agents. In the United States, recent terrorist incidents have resulted in the substantial enhancement of existing regulations and creation of new regulations governing laboratory security to prevent such incidents.

To facilitate the increase in regulation of biological research, the review and approval process for research utilizing biohazardous agents and recombinant DNA molecules must consider:

- Risk and threat assessment
- Facility security plans
- Physical security
- Data and electronic technology systems
- Security policies for personnel
- Policies regarding accessing the laboratory and animal areas

- Specimen accountability
- Receipt of agents into the laboratory
- Transfer or shipping of select agents from the laboratory
- Emergency response plans
- Reporting of incidents, unintentional injuries, and security breaches

Appendix A

Biological Safety Cabinets (BSCs)

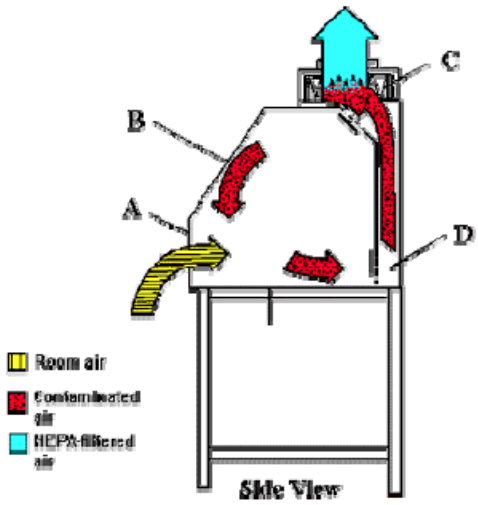
BSC's are designed to protect the operator, laboratory environment and work materials from exposure to infectious aerosols and splashes that may be generated when manipulating materials containing infectious agents, such as primary cultures, stocks and diagnostic specimens. When used properly, BSCs are highly effective in reducing laboratory-acquired infections and cross-contaminations of cultures due to the use of HEPA filters in the exhaust system.

Note: horizontal and vertical flow cabinets (e.g. a "clean bench") and chemical fume hoods cannot substitute for a BSC and a BSC, unless specially designed, cannot substitute for a chemical fume hood (see Table 5).

The risks to laboratory personnel and the environment are always the first consideration in determining the appropriate type of biological safety cabinet; however, the desired level of product protection must also be considered (see Table 6.)

Class I BSCs were the earliest design of biological safety cabinets intended to protect the laboratory worker from exposure to materials inside the cabinet.

Class I BSC

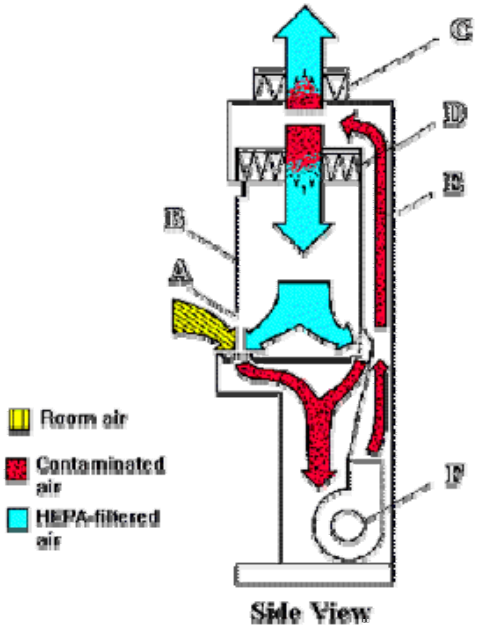


Room air is drawn into the cabinet and passes over the work surface. Airflow directs the air and any aerosols away from the laboratory worker and into the exhaust duct. Air from the cabinet is HEPA filtered into the laboratory and may be exhausted outside of the building through the building exhaust system or directly outside. Provides adequate personnel and environmental protection but does not provide reliable product production. In other words, cultures in use inside a Class I BSC are likely to become contaminated by materials inside and outside the cabinet.

Class II BSCs were designed to provide adequate product protection, as well as protection to the lab worker and the environment.

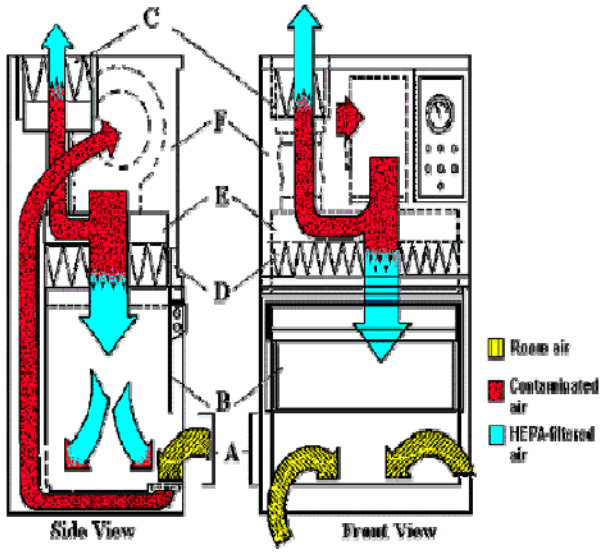
Class II A1 BSC

Room, or supply air passes through a HEPA filter before flowing downwards over the work surface. It then "splits": one half of the downwards flowing air passes through a front exhaust grill and the other half



Class II A2 BSC (formerly B3)

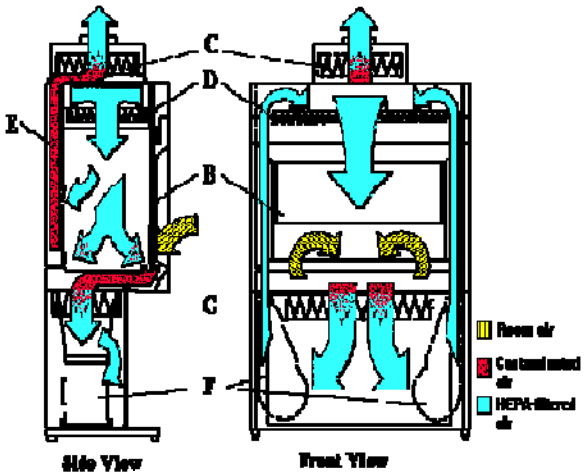
passes through a rear exhaust grill. The air, and any aerosols being carried in it, is discharged through the rear plenum into a space between the supply and exhaust filters, located at the top.
70% of the exhaust air is recirculated through the supply HEPA filter and back into the work zone. The remaining 30% passes through the exhaust HEPA filter and is recirculated to the room or outside, either via the building exhaust or through a thimble connection to a dedicated duct (see Class II, AII).



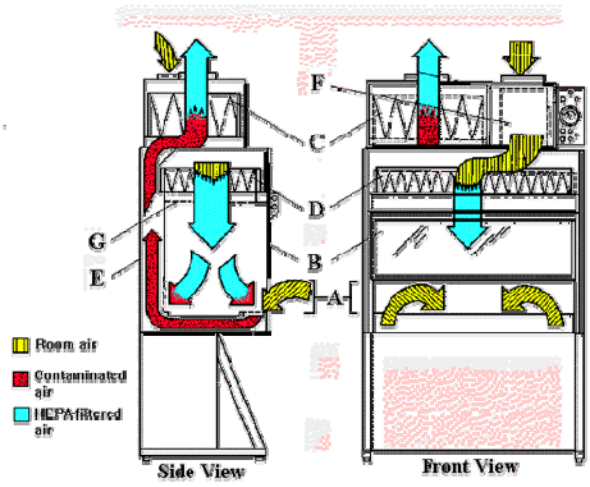
Room, or supply air passes through a HEPA filter before flowing downwards over the work surface. It then “splits”: one half of the downwards flowing air passes through a front exhaust grill and the other half passes through a rear exhaust grill. The air, and any aerosols being carried in it, is discharged through the rear plenum into a space between the supply and exhaust filters, located at the top.
70% of the exhaust air is recirculated through the supply HEPA filter and back into the work zone. The remaining 30% is exhausted outside through a thimble connection to a dedicated duct.

Class II B1 and BII biological safety cabinets are designed to meet higher containment needs, e.g. Biosafety Level 3 work. The exhaust must be hard ducted; they have a higher face velocity on the supply air; the B1 only recirculates 30% of the exhaust air inside the cabinet; and a BII does not recirculate any exhaust air.

Class II B1 BSC



Class II BII BSC



Class III BSCs are designed for total containment *with dedicated air supply and exhaust with no recirculation. This is appropriate for some BSL3 and all* BSL4 level work. For more information on this type of cabinet, please refer to the CDC *publication,* Primary Containment for Biohazards: Selection, Installation and Use of Biological Safety Cabinets, 2nd Edition *(2002).*

Table 5 – Comparison of BSC Characteristics

BSC Class	Face Velocity	Airflow Pattern	Applications	
			Non Volatile Toxic Chemicals and Radionuclides	Volatile Toxic Chemicals and Radionuclides
I	75	In at front; exhausted through HEPA to the outside or into the room through HEPA	YES	YES
II, A1	75	70% recirculated to the cabinet work area through HEPA; 30% balance can be exhausted through HEPA back into the room or to the outside through a thimble unit	YES	NO
II, A2	100	Same as II A1 but plenums are under negative pressure to room; exhaust air is thimble-ducted to the outside through a HEPA filter	YES	YES (minute amounts)
II, B1	100	Exhaust cabinet air must pass through a dedicated duct to the outside through a HEPA filter	YES	YES (minute amounts)
II, B2	100	No recirculation; total exhaust to the outside through hard-duct and a HEPA filter	YES	YES (small amounts)
III	N/A	Supply air inlets and hard-duct exhausted to outside through two HEPA filters in series	YES	YES (small amounts)

Table 6. Selection of a Safety Cabinet Through Risk Assessment

Biological Risk Assessed	Protection Provided			BSC Class
	Personnel	Product	Environmental	
BSL 1-3	YES	NO	YES	I
BSL 1-3	YES	YES	YES	II (A, B1, B2, B3)
BSL 4	YES	YES	YES	III

Appendix B

Classification of Human Pathogens on the Basis of Hazard

Biological agents that are known to infect humans are classified according to risk groups. The following listing of the more commonly encountered agents is reproduced from Appendix B of the NIH Guidelines. Included are representative genera and species known to be pathogenic; it is not meant to be all-inclusive. Those agents not listed in RG2 through RG4 are not automatically or implicitly classified in RG1.

Risk Group 1 (RG1) Agents

RG1 agents are not associated with disease in healthy adult humans. Examples of RG1 agents include asporogenic *Bacillus subtilis* or *Bacillus licheniformis*; adeno-associated virus (AAV) types 1 through 4; and recombinant AAV constructs, in which the transgene does not encode either a potentially tumorigenic gene product or a toxin molecule and are produced in the absence of a helper virus.
A strain of *Escherichia coli* is an RG1 agent if it (1) does not possess a complete lipopolysaccharide (i.e., lacks the O antigen); and (2) does not carry any active virulence factor (e.g., toxins) or colonization factors and does not carry any genes encoding these factors.
Those agents not listed in Risk Groups (RGs) 2, 3 and 4 are not automatically or implicitly classified in RG1; a risk assessment must be conducted based on the known and potential properties of the agents and their relationship to agents that are listed

Risk Group 2 (RG2) Agents

RG2 agents are associated with human disease which is rarely serious and for which preventive or therapeutic interventions are *often* available.
Risk Group 2 (RG2) - Bacterial Agents Including Chlamydia

--*Acinetobacter baumannii* (formerly *Acinetobacter calcoaceticus*)
--*Actinobacillus*
--*Actinomyces pyogenes* (formerly *Corynebacterium pyogenes*)
--*Aeromonas hydrophila*
--*Amycolata autotrophica*
--*Archanobacterium haemolyticum* (formerly *Corynebacterium haemolyticum*)
--*Arizona hinshawii* - all serotypes
--*Bacillus anthracis*
--*Bartonella henselae, B. quintana, B. vinsonii*
--*Bordetella* including *B. pertussis*
--*Borrelia recurrentis, B. burgdorferi*
--*Burkholderia* (formerly *Pseudomonas* species)
--*Campylobacter coli, C. fetus, C. jejuni*
--*Chlamydia psittaci, C. trachomatis, C. pneumoniae*

--*Clostridium botulinum, Cl. chauvoei, Cl. haemolyticum, Cl. histolyticum, Cl. novyi, Cl. septicum, Cl. tetani*
--*Corynebacterium diphtheriae, C. pseudotuberculosis, C. renale*
--*Dermatophilus congolensis*
--*Edwardsiella tarda*
--*Erysipelothrix rhusiopathiae*
--*Escherichia coli* - all enteropathogenic, enterotoxigenic, enteroinvasive and strains bearing K1 antigen, including *E. coli* O157:H7
--*Haemophilus ducreyi, H. influenzae*
--*Helicobacter pylori*
--*Klebsiella* - all species except *K. oxytoca* (RG1)
--*Legionella* including *L. pneumophila*
--*Leptospira interrogans* - all serotypes
--*Listeria*
--*Moraxella*
--*Mycobacterium* including *M. avium* complex, *M. asiaticum, M. bovis* BCG vaccine strain, *M. chelonei, M. fortuitum, M. kansasii, M. leprae, M. malmoense, M. marinum, M. paratuberculosis, M. scrofulaceum, M. simiae, M. szulgai, M. ulcerans, M. xenopi*
--*Mycoplasma*, except *M. mycoides* and *M. agalactiae* which are restricted animal pathogens
--*Neisseria gonorrhoeae, N. meningitidis*
--*Nocardia asteroides, N. brasiliensis, N. otitidiscaviarum, N. transvalensis*
--*Rhodococcus equi*
--*Salmonella* including *S. arizonae, S. cholerasuis, S. enteritidis, S. gallinarum-pullorum, S. meleagridis, S. paratyphi,* A, B, C, *S. typhi, S. typhimurium*
--*Shigella* including *S. boydii, S. dysenteriae,* type 1, *S. flexneri, S. sonnei*
--*Sphaerophorus necrophorus*
--*Staphylococcus aureus*
--*Streptobacillus moniliformis*
--*Streptococcus* including *S. pneumoniae, S. pyogenes*
--*Treponema pallidum, T. carateum*
--*Vibrio cholerae, V. parahemolyticus, V. vulnificus*
--*Yersinia enterocolitica*

Risk Group 2 (RG2) - Fungal Agents

--*Blastomyces dermatitidis*
--*Cladosporium bantianum, C. (Xylohypha) trichoides*
--*Cryptococcus neoformans*
--*Dactylaria galopava (Ochroconis gallopavum)*
--*Epidermophyton*
--*Exophiala (Wangiella) dermatitidis*
--*Fonsecaea pedrosoi*
--*Microsporum*
--*Paracoccidioides braziliensis*
--*Penicillium marneffei*

--*Sporothrix schenckii*
--*Trichophyton*

Risk Group 2 (RG2) - Parasitic Agents

--*Ancylostoma* human hookworms including *A. duodenale, A. ceylanicum*
--*Ascaris* including *Ascaris lumbricoides suum*
--*Babesia* including *B. divergens, B. microti*
--*Brugia* filaria worms including *B. malayi, B. timori*
--*Coccidia*
--*Cryptosporidium* including *C. parvum*
--*Cysticercus cellulosae* (hydatid cyst, larva of *T. solium*)
--*Echinococcus* including *E. granulosis, E. multilocularis, E. vogeli*
--*Entamoeba histolytica*
--*Enterobius*
--*Fasciola* including *F. gigantica, F. hepatica*
--*Giardia* including *G. lamblia*
--*Heterophyes*
--*Hymenolepis* including *H. diminuta, H. nana*
--*Isospora*
--*Leishmania* including *L. braziliensis, L. donovani, L. ethiopia, L. major, L. mexicana, L. peruvania, L. tropica*
--*Loa loa* filaria worms
--*Microsporidium*
--*Naegleria fowleri*
--*Necator* human hookworms including *N. americanus*
--*Onchocerca* filaria worms including, *O. volvulus*
--*Plasmodium* including simian species, *P. cynomologi, P. falciparum, P. malariae, P. ovale, P. vivax*
--*Sarcocystis* including *S. sui hominis*
--*Schistosoma* including *S. haematobium, S. intercalatum, S. japonicum, S. mansoni, S. mekongi*
--*Strongyloides* including *S. stercoralis*
--*Taenia solium*
--*Toxocara* including *T. canis*
--*Toxoplasma* including *T. gondii*
--*Trichinella spiralis*
--*Trypanosoma* including *T. brucei brucei, T. brucei gambiense, T. brucei rhodesiense, T. cruzi*
--*Wuchereria bancrofti* filaria worms

Animal Viral Etiologic Agents in Common Use

The following list of animal etiologic agents is appended to the list of human etiologic agents. None of these agents is associated with disease in healthy adult humans; they are commonly used in laboratory experimental work. A containment level appropriate for

RG1 human agents is recommended for their use. For agents that are infectious to human cells, e.g., amphotropic and xenotropic strains of murine leukemia virus, a containment level appropriate for RG2 human agents is recommended.
Baculoviruses
Herpesviruses Herpesvirus ateles Herpesvirus saimiri Marek's disease virus Murine cytomegalovirus
Papovaviruses Bovine papilloma virus Polyoma virus Shope papilloma virus Simian virus 40 (SV40)
Retroviruses Avian leukosis virus Avian sarcoma virus Bovine leukemia virus Feline leukemia virus Feline sarcoma virus Gibbon leukemia virus Mason-Pfizer monkey virus Mouse mammary tumor virus Murine leukemia virus Murine sarcoma virus Rat leukemia virus

Murine Retroviral Vectors

Murine retroviral vectors to be used for human transfer experiments (less than 10 liters) that contain less than 50% of their respective parental viral genome and that have been demonstrated to be free of detectable replication competent retrovirus can be maintained, handled, and administered, under BL1 containment.

Appendix C

Chemical Decontamination

Definitions:

Antimicrobial	An agent that kills microorganisms or suppresses their growth and multiplication.
Antisepsis	A germicide applied to living tissue or on the skin for the purpose of inhibiting or destroying microorganisms.
Biocide	A general term for any agent that kills unicellular and multicellular organisms.
Decontamination	Destruction or removal of microorganisms to an acceptable lower level, not necessarily zero. This also applies to removal or neutralization of toxic agents and, in microbiological laboratories, implies microbiocidal action for safety purposes
Disinfection	The use of chemical or physical treatment that destroys most vegetative microbes (or viruses), but not spores, in or on inanimate objects.
Sanitization	Reduction of microbial load on an inanimate surface to a "safe" public health level.
Sterilization	The use of a physical or chemical procedure to perform total destruction of all living organisms, microorganisms, including large numbers of highly resistant bacterial spores. In practice, sterility is very difficult to determine. Therefore, the term is applied to a very low probability (e.g., usually 10-6) that even one organism has survived the process.

Decontamination Methods

Physical and chemical decontamination fall into four main categories:

- heat
- vapors and gases
- radiation
- liquid decontaminants

Wet Heat

Autoclaving (saturated steam under pressure, 15-18 psi, to achieve a chamber temperature of 121-132°C (250-270°F) for a prescribed time) is the most convenient method of rapidly achieving destruction of all forms of microbial life. Attention must be paid to the loading of the autoclave to prevent overloading and entrapment of air.

Materials to be autoclaved must come into contact with the steam and heat. Chemical indicators such as autoclave tape may be used with each load but it is not an adequate monitor of efficacy. Autoclave sterility monitoring must be conducted on a monthly basis with appropriate biological indicators such as *Bacillus stearothermophilus* spore strips place at appropriate locations throughout the autoclave. The spores, which can survive 250°F for five minutes but are killed in 13 minutes, are more resistant to heat than most, thereby providing an adequate margin of safety when validating sterility. Vet Services is currently using biological indicators to monitor all autoclaves in the research building. ***Autoclaving is not considered a final treatment for waste disposal at the University of Cincinnati..***

Laboratory employees should be cautious because steam under pressure can be a source of scalding jets if the equipment for its application is mishandled. Loads of manageable size should be used. Fluids treated by steam under pressure may be superheated if removed from the sterilizer immediately after treatment. This can cause a sudden and violent boiling of the contents from containers that can splash scalding liquids onto personnel handling the containers. Dry hypochlorites (bleach), or any other strong oxidizing material, must not be autoclaved with organic material such as paper, cloth or oil.

OXIDIZER + ORGANIC MATERIAL + HEAT = POSSIBLE EXPLOSION

Dry Heat

Dry heat is less efficient than wet heat and requires longer times and/or higher temperatures to achieve sterilization. It is suitable for the destruction of microorganisms on impermeable non-organic surfaces such as glass but is not reliable in the presence of thin layers of organic materials which may act as insulation. Sterilization of glassware by dry heat can usually be accomplished at 160-170°C for periods of 2-4 hours. Dry heat should also be monitored with biological indicators (spore strips).

Incineration

Incineration is another effective means of decontamination by heat and is useful for disposal of biohazardous waste as it also reduces the volume of material. Incineration is the method of choice for disposal of animal carcasses and pathology specimens. However, there are no incinerators here at CCHMC. All CCHMC biohazard waste to be incinerated is sent out in specially marked containers.

Vapors and Gases

A variety of gases and vapors possess germicidal properties. The most useful of these are formaldehyde and ethylene oxide. When these are employed in closed systems and under controlled conditions of temperature and humidity, excellent results can be obtained. Vapor and gas decontaminants are primarily useful in decontaminating biological safety cabinets and associated air handling systems and air filters; bulky or stationary equipment that resists penetration by liquid surface decontaminants; instruments and optics that may

be damaged by other decontamination methods; and rooms, buildings and associated air-handling systems.

Because both ethylene oxide and formaldehyde exposure must be monitored as required by OSHA, these are not options for the general laboratory. Mutagenic potential has been attributed to ethylene oxide; toxic effects, hypersensitivity and possible carcinogenic effects are well-documented for formaldehyde. Ethylene oxide use is limited and is generally used in surgical and clinical areas at CCHMC. Contact Health and Safety (xxxx) for more information regarding these methods.

Radiation

Ionizing radiation (gamma and X-ray) will destroy microorganisms and has been used for sterilization of prepackaged medical devices, however, it is not a practical tool for laboratory use. Non-ionizing radiation, such as ultraviolet radiation (UV), is used for inactivating viruses, mycoplasma, bacteria and fungi. It will destroy airborne microorganisms and inactivate microorganisms on exposed surfaces, or in the presence of products of unstable composition that cannot be treated by conventional means.

The usefulness of UV radiation as a sanitizer is limited by its low penetrating power. Microorganisms inside dust or soil particles will be protected from its action, limiting its usefulness. UV radiation is primarily used in air locks, animal holding areas, ventilated cabinets and in laboratory rooms during unoccupied periods to reduce the levels of viable airborne microorganisms and to maintain good air hygiene. Because UV radiation can cause burns to the cornea and skin if exposed for even a short period of time, proper shielding should be used. UV lamps that are used for space decontamination should be interlocked with the general room or cabinet illumination so that turning on the lights extinguishes the UV light. Post the area with warning signs if UV lights are to be left on.

UV lamps are not recommended for decontamination unless they are properly maintained. Because UV lamp intensity or destructive power decreases with time, it should be checked with a UV meter yearly. Frequent cleaning of the UV bulb every few weeks is necessary to prevent accumulation of dust and dirt which can drastically reduce the effectiveness.

Liquid Disinfectants

The most practical use of liquid disinfectants is for surface decontamination and to decontaminate liquid wastes prior to final disposal in the sanitary sewer (please see note under ‘chlorine’ and under ‘disposal’). Liquid disinfectants are widely available under a variety of trade names. In general they can be classified as alcohols, phenolic compounds, quaternary ammonium compounds (Quats), aldehydes, acids or alkalines, amines and halogens. The most effective disinfectants are frequently very corrosive and toxic. None are equally useful or effective under all conditions for all infectious agents.

Particular care should be observed when handling concentrated stock solutions of disinfectants. Personnel making up use-concentrations from stock solutions must be informed of the potential hazards, trained in the safe procedures to follow and appropriate

personal protective equipment to use as well as the toxicity associated with ocular, skin and respiratory exposure.

Properties of Some Common Liquid Decontaminants

Alcohol
Ethyl or isopropyl alcohol are good general-use disinfectants that are active against vegetative bacteria, fungi and lipid-containing viruses but not against spores. Their action against non-lipid viruses is variable. For highest effectiveness they should be used at concentrations of 70% (v/v) in water for 10 minutes (might require repeated application); higher concentrations are not as effective (100% actually dehydrates and may preserve the microorganisms). A major advantage of aqueous solutions of alcohol is that they do not leave any residue on treated surfaces. However, they are flammable and evaporate rapidly, and therefore have limited exposure time.

Chlorine
Chlorine solutions are universal decontaminants active against many microorganisms including bacterial spores. Solutions are fast acting and have broad spectrum activity. Sodium hypochlorite is the most common base for chlorine disinfectants. Common household bleach can be diluted 1:10 to yield a satisfactory disinfectant solution (5 g/l available chlorine) with a contact time of 10 - 30 minutes. It is a strong oxidizing agent and corrosive to metals. Its activity is greatly reduced by organic matter so surfaces must be clean before using as a disinfectant. Chlorine solutions must be stored capped and prepared frequently (at least weekly for general use, right before use for spills) as the chlorine tends to off-gas from the solution. Chlorine gas is highly toxic. Bleach must never be mixed with acids in order to avoid the rapid release of chlorine gas. Many by-products of chlorine can be harmful to humans and the environment so that indiscriminate use of chlorine-based disinfectants, and in particular bleach, should be avoided.

An acceptable way to decontaminate liquid biohazardous waste from RG 2 agents here at CCHMC is to add bleach to 15% (v/v), let the mixture sit for at least 20 minutes and then dispose down the sanitary sewer system.

Formaldehyde / Formalin
Formaldehyde is usually marketed as a solution of about 37% concentration. Dilution of formaldehyde to 5% results in an effective disinfectant that inactivates vegetative bacteria, bacterial spores, lipid and nonlipid viruses and fungi. The odor, irritation to skin and eyes and toxicity of formaldehyde solutions reduce its desirability for general use. Formaldehyde solutions are active in the presence of organic matter. Formaldehyde is an OSHA-regulated chemical and is not recommended for disinfection purposes at CCHMC. ***Formaldehyde is a suspected human carcinogen and creates respiratory problems at low levels of concentration***.

Glutaraldehyde

Glutaraldehyde is effective against all types of bacteria, fungi and viruses. It is non-corrosive and faster acting than formaldehyde, however, it takes several hours to kill bacterial spores. It is generally supplied as a solution with a concentration of about 20 g/l and most products need to be activated (made alkaline) before use. The activated solution can be reused for 1-4 weeks depending on the type and frequency of use. Glutaraldehyde is frequently used to disinfect medical instruments that cannot be autoclaved. Glutaraldehyde is toxic and an irritant to skin, mucous membranes, and upper respiratory tract. It should always be used in a chemical fume hood with the proper PPE.

Iodine and Iodophors

Iodine solutions or tinctures have long been used primarily as antiseptics on skin or tissue. Iodophors are combinations of iodine and a solubilizing agent or carrier. The action of these disinfectants is similar to that of chlorine but may be slightly less affected by organic matter. A peculiarity of iodophors is the reverse relationship between the concentration and bacteriocidal activity; the more concentrated the iodophor, the less activity it has. The germicidal activity is dependent upon release of free iodine from the compound in which it is contained. At high concentrations the iodophor complexes to form micells and is not available for disinfection, therefore, it is important to follow the manufacturer's recommendations for dilution. Iodophors are good surface disinfectants but are not recommended for the disinfection of liquids.

Phenol and Phenol Derivatives

Phenol itself is not widely used as a decontaminant due to its strong odor, toxicity and a sticky residue that remains on the surface. Phenolic compounds and phenol homologs normally used in a 5-10% concentration range are basic to a number of popular disinfectants. Phenols are effective against vegetative bacteria, fungi and lipoviruses. It is not effective against bacterial spores and non-lipid viruses.

Quaternary Ammonium Compounds (Quats)

Quats are cationic detergents with strong surface activity. They are acceptable for low level, general-use disinfectants and are active against Gram-positive bacteria and lipid-containing viruses. They are less active against Gram-negative bacteria and are not active against non-lipid viruses. Quats are easily inactivated by organic materials, anionic detergents or salts of metals found in water. They are more active when used in mixtures with phenols or alcohols. However, they may accumulate in the environment due to their low biodegradability.

General Decontamination Guidelines

Because the specific requirements for decontamination will depend upon the work and the nature of the organism involved, the initial risk assessment for laboratory work shall include an evaluation of the processes and agents to be used to ensure that the biohazardous materials involved are inactivated during daily disinfection of laboratory surfaces, periodic equipment cleaning, spill clean-up, and upon final disposal.

The CDC's Biosafety in Microbiological and Biomedical Laboratories (BMBL), the NIH Guidelines for Research Involving Recombinant DNA Molecules and the OSHA Bloodborne Pathogens Standard (CFR 1910.1030) all require disinfection of contaminated work surfaces after the completion of the procedure, immediately or as soon as is feasible after any overt contamination of surfaces or any spill of potentially infectious material and/or at the end of the work shift. Equipment shall be decontaminated periodically and immediately or as soon as is feasible after visible contamination.

Since many liquid disinfectants are inactivated by the presence of organic matter and thus dirty items cannot be efficiently disinfected or sterilized, it is important to understand the fundamentals of pre-cleaning. In order to disinfect a surface or piece of equipment, it must first be pre-cleaned. In practical terms, cleaning is the removal of visible dirt and stains. This is generally achieved either by (a) brushing, vacuuming or dry dusting; or (b) washing or damp mopping with water containing a soap or detergent. Pre-cleaning is essential to achieve proper disinfection or sterilization because dirt and soil can shield microorganisms and can also interfere with the killing action of chemical germicides. Also, many germicidal products claim activity only on pre-cleaned items. Pre-cleaning must be carried out with care to avoid exposure to infectious agents, and materials chemically compatible with the germicides to be applied later must be used. It is quite common to use the same chemical germicide for pre-cleaning and disinfection.

The appropriate liquid disinfectant should be chosen after careful consideration of many factors:

- Biohazardous agent – Risk assessment.
- Degree of contamination – This will affect the time required for disinfection and the amount of disinfectant.
- Presence of organic material - The proteins in organic materials such as blood, bodily fluids, and tissue can prevent or slow the activity of certain disinfectants. It is important to clean a surface area prior to disinfection.
- Nature of surface being disinfected - Porous or smooth; the more porous and rough the surface, the longer a disinfectant will need in order to be effective.
- Nature of the disinfectant – Not all disinfectants are able to inactivate all microorganisms and their spores.
- Duration of exposure, temperature, and pH - Increased exposure time increases the effectiveness of disinfectants. Low temperatures may slow down the activity requiring more exposure time
- Residual activity of the disinfectant and effects on fabric and metal
- Toxicity to the environment and relative safety to animals that may be exposed.

Table 1. Increasing Resistance to Chemical Disinfectants

Least Resistant	Agents	Examples	Level of Disinfection Required
	Lipid Viruses or Medium-size Viruses	Herpes simplex virus Cytomegalovirus Respiratory synctial virus Hepatitus B virus Human Immunodeficiency Virus	Low level
	Vegetative Bacteria	*Pseudomonas aeruginosa* *Staphylococcus aureus* *Salmonella choleraesuis*	
	Fungi	*Trichophyton* sp. *Cryptococcus* sp. *Candida* sp	
	Non-Lipid Viruses or Small Viruses	Poliovirus Coxsackievirus Rhinovirus	Intermediate Level
	Mycobacteria	*Mycobcterium tuberculosis* *M. bovis*	High Level
Most Resistant	Bacterial Spores	*Bacillus subtilis* *Clostridium sporogenes*	Sterilization